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Evaluation of a Wettable Powder Formulation for the Nuclear Polyhedrosis Virus of *Anticarsia gemmatalis* (Lep.: Noctuidae)

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Abstract: The influence of different carriers on the physical and biological stability of *Baculovirus anticarsia* wettable powder formulations was studied. The formulations were obtained by mixing the purified polyhedra of *B. anticarsia* with a carrier and drying the suspension in a bench spray dryer. Bioassays with *Anticarsia gemmatalis* Hübner showed that activity was maintained with the amorphous silica, attapulgite and kaolinite after a year of storage. In the presence of bentonite, activity declined 50% in the same period. All formulations, except kaolinite, maintained the physical parameters required of a good wettable powder. Kaolinite formulation showed reduction in wettability and particle agglomeration in storage.

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1 INTRODUCTION

Viral insecticides have typically been formulated as wettable powders for application as sprays with conventional equipment.[1] Several properties have to be considered when formulating wettable powders: flowability, wettability, dispersibility, suspensibility, foaming, and both physical and chemical storage stability. Most of these properties are concerned with the ability of the product to form a homogeneous sprayable suspension.[2] The rate of sedimentation in the spray tank is directly proportional to size squared and density of the particles.[3] Spray drying has proved to be the most successful approach for producing stable nuclear polyhedrosis virus (NPV) formulations with fine particles. In a recent paper, different methods to obtain dry preparations were compared.[4] Another important aspect in the development of a wettable powder is appropriate carrier selection. The effect of long-term storage on the activity of NPVs has not been adequately studied.[5] The objective of this work was to study the influence of different carriers on the physical and biological stability of *Baculovirus anticarsia* (NPV of *Anticarsia gemmatalis* Hübner) wettable powder formulations.

2 MATERIALS AND METHODS

2.1 Wettable powder preparations

The formulations were prepared with four different carriers: kaolinite (two-layer clay 1 : 1, Ouro Branco); bentonite (a three-layer type 2 : 1, Viscogel/Blanver); attapulgite (a chain type, Attagel 50/Adexin); and an amorphous silica (Aerosil 200/Degussa). The kaolinite was submitted to colloidal milling for one hour. Other

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carriers were used as received. Each sample was suspended in distilled water and submitted to agitation in an Ultra-Turrax for 20 min. The pH of the suspensions was adjusted to *c.*5·5 with hydrochloric acid. The wettable powders were prepared by mixing the polyhedra of *B. anticarsia* with carrier (1 + 1 dry weight) and drying in a Buchi Mini Spray Dryer under the following conditions: spray performance, 980 ml h^{-1}; inlet temperature, 125°C; solids concentration, 30 g $litre^{-1}$. The dry samples were stored in the dark at room temperature in a bottle sealed with a rubber stopper.

2.2 Particle size and wettability

The particle size of each wettable powder was measured using a Malvern 3600 laser diffraction particle sizer, recommended for the range from 1 to 1800 μm. Mechanical agitation was applied to the samples prior to measurements of particle sizes, and the relative and cumulative relative frequency for each class size and the 10th, 50th and 90th percentiles were calculated. Wettability was determined by pouring 60 mg of each powder upon the surface of a serial dilution of ethanol and water (10% steps). The tests were performed at 25(±1)°C.[6]

2.3 *Baculovirus anticarsia* purification

Fifth-instar larvae of *A. gemmatalis* reared on an artificial diet at 28°C were infected with a suspension of 10^7 polyhedron inclusion bodies (PIB) ml^{-1}. Six to ten days after infection, the larvae with nucleopolyhedrosis symptoms were frozen at −18°C. The PIB were purified using a modification of the method proposed by Van der Geest.[7] After defrosting, the larvae were left to decompose and the preparation was filtered through synthetic fabric and diluted to a concentration of 10 g dm^{-3} of solids. The suspension was centrifuged at 12 000 rev min^{-1} and the solids were resuspended in water.

2.4 Bioassay

Each formulation was suspended in distilled water, and 5 μl was applied to the surface of the diet (38 mm^2), corresponding to 90 PIB per cup where each larva was maintained. A Neubauer haemocytometer was used to count the polyhedra, after submitting the sample to 1 h ultrasonic radiation to break the clumps. The samples used in the bioassays were only agitated sufficiently to simulate normal field conditions in a spray tank. One third-instar larva was placed in each 50-ml cup containing the inoculated diet where it remained for 48 h, and then transferred to a polyhedron-free diet. Two controls were utilized: distilled water and a filtered extract of infected *A. gemmatalis*. The tests were performed with 60 larvae per treatment. Mortality was initially assessed daily and recorded until pupation or death and then corrected according to Abbot's formula.[8]

2.5 Statistical analysis

The expected effect of storage is that biological activity will remain constant or decrease with time. When reduction in mortality occurs, the rate of reduction is greater in the initial months, tending to stabilize afterwards. A negative and significant ($P < 0{\cdot}01$) slope estimate indicates that biological activity declines during storage. Bioassays were performed at different periods of storage. Linear regressions of percentage mortality on the natural logarithm of days of storage were fitted for each formulation. The regression parameters were estimated by the weighed least squares method.[9] The one-sided test was used to verify the hypothesis that mortality is constant (slope equals zero) or decreases with time (slope < zero).[10]

3 RESULTS AND DISCUSSION

Attapulgite, bentonite and amorphous silica showed similar patterns before and after storage, while kaolinite changed drastically in size distribution after storage. The percentage of particles less than 4 μm in all formulations was more than 95 after 10 months of storage, except for kaolinite, for which the percentage of particles smaller than 44 μm dropped from 100 to 55 after 10 months. Table 1 shows the percentiles (10th, 50th and 90th) and the difference between the 10th and 90th percentiles, the interpercentile range (IPR). The IPR for kaolinite changed from 1·7 μm to 80·2 μm, pointing to a drastic reduction in the uniformity of particle sizes after storage. For attapulgite there was a large change in the opposite direction. As regards bentonite, amorphous silica and *Baculovirus anticarsia* without carrier, small changes occurred in IPR.

The critical surface tension (γ_{crit}) for the immersion of formulations after storage was 46·1, 46·1, 38·5 and 34·0 mN m^{-1} for attapulgite, bentonite, amorphous silica and kaolinite, respectively. The immersion process is closely related to the wetting behaviour of the solids. It is possible to use the immersion experiments to evaluate the hydrophobicity of solid surfaces by measuring the surface tension for which immersion becomes spontaneous in mixtures of solvents.[6] The γ_{crit} of kaolinite (34·0 mN m^{-1}) indicates loss of wettability. In practice, this means that this powder stays on the surface of the water for an indefinite time and practically does not wet. The other formulations did not lose wettability after 10 months of storage.

Attapulgite, bentonite and amorphous silica formulations proved to be suitable wettable powders. Such powders must have a high proportion of particles less

TABLE 1
Sample Percentiles (10th, 50th and 90th) and Differences between 10th and 90th Percentiles (IPR) of Particle Size Distribution for Each Formulation of NPV of *Anticarsia gemmatalis*

	Percentile (μm)							
	10		*50*		*90*		*IPR* (μm)	
Carriers	(*i*)[a]	(*ii*)	(*i*)	(*ii*)	(*i*)	(*ii*)	(*i*)	(*ii*)
Attapulgite	2·9	3·0	7·6	5·7	80·9	11·2	78·0	8·2
Bentonite	2·7	2·9	6·5	6·7	20·9	29·3	18·2	26·4
Kaolinite	2·6	4·5	3·4	33·4	4·3	84·7	1·7	80·2
Amorphous silica	2·6	3·0	6·5	6·6	17·3	20·1	14·7	17·1

[a] (i) after preparation; (ii) after 10 months storage.

TABLE 2
Mean Mortalities of *Anticarsia gemmatalis* NPV Formulations that Maintained Nearly Constant Biological Activities during the Observation Period. Weighed Least Squares Estimates of Linear Regression Slopes, *t*-statistic for Hypothesis that it is Zero (Biological Stability) and Corresponding Type I Error, for Formulations of NPV of *Anticarsia gemmatalis* with Different Carriers

Carrier	*Mortality* (%)($\pm SE$)	*Total bioassays*	*Parameter estimate* ($\pm SE$)	*T-value for* H_o: $\beta = 0$	$Pr < -T$
Attapulgite	79·4 ± (6·9)	3	4·2 ± (1·9)	2·21	0·8647
Bentonite			−7·7 ± (0·3)	−23·88	0·0001[a]
Kaolinite	60·3 ± (7·2)	3	4·4 ± (0·5)	8·37	0·9621
Amorphous silica	83·1 ± (3·0)	5	3·5 ± (3·4)	1·04	0·8117
Baculovirus anticarsia without carrier	80·9 ± (4·9)	6	−7·7 ± (2·5)	−3·09	0·0135

[a] Statistically significant at 1% level.

than 5 μm and all should pass a 44 μm screen.[11] These conditions ensure that the formulations will remain uniformly suspended and free of nozzle-plugging particles in the mixing tank throughout the application period.[3] While finely divided particles are desirable, a narrow particle-size distribution (expressed as the IPR value) is also important, positively affecting the performance of products in the field.[2] Kaolinite showed a drastic reduction in the uniformity of particle size after storage, indicating that it was the worst carrier tested in relation to physical stability.

Table 2 shows the results of statistical analysis of the effect of storage on larval mortality. This was significantly reduced ($P < 0{\cdot}08$) from 90% to 45% after a year for the bentonite formulation. Amorphous silica showed the highest biological activity estimate (83·1% mortality) and lowest standard error. The poor physical performance of kaolinite accounts, at least partially, for the weakest biological activity. The results showed that the carrier selected has a marked influence on biological activity during storage. Other authors showed a higher reduction in the activity of NPV formulation after storage at ambient temperature than the results of this paper.[5,12–14] Several physicochemical properties of carriers can be responsible for the biological performance of formulation.[15] The good performance of amorphous silica leads one to suppose that this carrier maintains the polyhedra at adequate humidity levels after spray drying. Also, its low chemical reactivity compared to clays seems to be appropriate in the maintenance of biological activity.

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